In the Claims

We claim:

Claim 1 (Currently amended): A method for increasing the number of polynucleotides containing sequences corresponding to a mRNA species present in a sample, the method comprising the steps of:

(i) reverse ~~transcription of~~ transcribing the mRNA species using a heeled 5'-amplification primer (FAP-RAND) and a heeled 3'-amplification primer (TAP-RT), wherein each primer sequence is unique, and either or each heel sequence includes a RNA polymerase promoter site, and the FAP includes a variable sequence, whereby the RNA is reverse-transcribed to produce double-stranded cDNA and then multiple cDNAs according to the variable sequence; and

(ii) ~~amplification of~~ amplifying the cDNA using primers sufficiently complementary to the ~~primers, *i.e.*,~~ primer sequences FAP and TAP, within FAP-RAND and TAP-RT.

Claim 2 (Currently amended): ~~A~~ The method according to claim 1, which additionally comprises the step of:

(iii) *in vitro* ~~transcription~~ transcribing, to produce RNA run-offs from either end of the amplicons.

Claim 3 (Currently amended): ~~A~~ The method according to claim 1 ~~or claim 2~~, wherein each heel sequence includes a different RNA polymerase site.

Claim 4 (Currently amended): ~~A~~ The method according to claim 3, for the production of a strand-specific library.

Claim 5 (Currently amended): ~~A~~ The method according to ~~any preceding claim~~ claim 1, for the production of a subtracted library from two cell populations.

Claim 6 (Currently amended): ~~A~~ The method according to ~~any preceding claim~~ claim 1, which further comprises cloning the polynucleotide products and immobilizing them in an array.

Claim 7 (Currently amended): ~~A~~ The method according to ~~any preceding claim~~ claim 1, wherein the sample is from laser capture microdissection.

Claim 8 (Currently amended): ~~A~~ The method according to ~~any preceding claim~~ claim 1, wherein the sample is from patch clamp harvesting.

Claim 9 (Currently amended): ~~A~~ The method according to ~~any preceding claim~~ claim 1, wherein the first ~~and/or~~ or the second heel sequence, or both, includes the nucleotide sequence of a cleavage site.

Claim 10 (Currently amended): ~~A~~ The method according to claim 9, wherein the cleavage site is located at the 3' end of its heel sequence.

Claim 11 (Currently amended): ~~A~~ The method according to claim 10, wherein the first and second heeled primers have identical cleavage sites.

Claim 12 (Currently amended): ~~A~~ The method according to claim 10, wherein the first and second heeled primers have different cleavage sites.

Claim 13 (Currently amended): ~~A~~ The method according to ~~any of claims 9 to 12~~ claim 9, which comprises the additional step of treating the polynucleotides with an agent that cleaves at the cleavage site.

Claim 14 (Currently amended): ~~A~~ The method according to ~~any preceding claim~~ claim 1, wherein ~~amplification~~ said amplifying comprises up to 50 amplification cycles.

Claim 15 (Currently amended): ~~A~~ The method according to claim 14, wherein each amplification cycle comprises the steps of:

(i) obtaining single-stranded DNA molecules at a temperature between 85°C and 97°C;

(ii) annealing the single-stranded DNA molecules at a temperature between 45°C and 65°C; and

(iii) elongating the annealed DNA molecules at a temperature between 70°C and 75°C.

Claim 16 (Currently amended): ~~A~~ The method according to ~~any preceding claim~~ claim 1, wherein the first heeled primer population consists of a population of nucleic acids comprising, from 5' end to 3' end:

(i) a heel sequence, of 15 to 22 nucleotides, which is not complementary to the mRNA molecules initially present in the sample; and

(ii) an oligo dT sequence of 15 to 25 nucleotides;

wherein substantially every possible variable sequence combination is found in said first heeled primer population.

Claim 17 (Currently amended): ~~A~~ The method according to ~~any preceding claim~~ claim 1, which additionally comprises confirming the presence of at least one nucleic acid sequence contained in the reaction mixture after ~~amplification~~ said amplifying.

Claim 18 (Currently amended): ~~A~~ The method according to claim 17, wherein ~~the~~ said confirming comprises any ~~one of~~ of the following methods:

(i) ~~detection of~~ detecting sequences of interest with specific oligonucleotide probes;

(ii) ~~amplification of~~ amplifying sequences of interest with specific oligonucleotide primers; and

(iii) cloning ~~of the~~ DNA molecules obtained in a replication ~~and/or~~ or expression vector.